Kidney International, Vol. 56, Suppl. 71 (1999), pp. S-256–S-259

Role of glomerular epithelial cell-derived heat shock protein 47 in experimental lipid nephropathy

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Role of glomerular epithelial cell-derived heat shock protein 47 in experimental lipid nephropathy.

Background. Heat shock protein 47 (hsp47) is a collagen-specific stress protein and is shown to be involved in the synthesis/assembly of various collagens as a molecular chaperone. This study was undertaken to investigate the possible role of hsp47 in dietary-induced hypercholesterolemic rat kidneys, which showed glomerular hypercellularity with expansion of mesangial matrix.

Methods. Dietary-induced hypercholesterolemia was induced in male Wistar rats by giving 2% cholesterol diet for four months. Immunohistochemistry was used for localization of protein products for collagens (types I, III, and IV), α-smooth muscle actin, vimentin, desmin, and ED-1, a macrophage/monocyte marker, and hsp47 in control and hypercholesterolemic rat kidneys.

Results. Compared with the control, increased accumulation of collagens was accompanied with increased expression of hsp47 in hypercholesterolemic rat kidneys, with predominant expression in the glomeruli. By double immunostaining, desmin-positive glomerular epithelial cells were found to be the main source of hsp47 in hypercholesterolemic rat kidneys.

Conclusion. From these results, it is concluded that induced expression of hsp47 by phenotypically altered glomerular epithelial cells might play a role in the excessive assembly/synthesis of collagens and could thereby contribute to the glomerulosclerosis found in dietary-induced hypercholesterolemic rat kidneys.

Previous studies have shown that long-term dietary-induced hypercholesterolemia results in glomerular injury, including mild glomerular hypercellularity, expansion of mesangial matrix, glomerular infiltration of foam cells, and inflammatory cell infiltration, which leads to mild renal dysfunction and proteinuria [1, 2]. Furthermore, it has been reported that glomerulosclerosis in high-cholesterol–fed rats is associated with increased glomerular accumulation of extracellular matrix, including type IV collagen [2]. Although morphological changes in lipid-induced glomerular injury are reported elsewhere [1, 2], the exact molecular mechanisms of these changes are not yet clear.

Heat shock protein 47 (hsp47) is a stress protein that specifically binds newly synthesized procollagen in the endoplasmic reticulum and is shown to have a role in folding and assembling procollagen molecules as a collagen-specific molecular chaperone [3]. Recent studies suggested a possible pathophysiological role of hsp47 in sclerotic/fibrotic process in various organs [4–8]; its expression was augmented in association with increased deposition of collagens in experimental liver cirrhosis and pulmonary fibrosis [5, 6]. Induced expression of hsp47 by renal cells was also noted in experimental renal sclerotic/fibrotic kidneys [4, 7]. Because abnormal collagen metabolism may also contribute to glomerulosclerosis by a high-fat diet [2], hsp47 may play a role in high-fat diet-induced glomerular injury. In this study, the possible role of hsp47 in high-cholesterol diet-fed rat kidneys was investigated.

METHODS

Experimental design

Male Wistar rats ($N = 14$) aged eight weeks were divided into two experimental groups. Group I ($N = 5$) consisted of age-matched control rats that were fed with normal pelleted diet. Group II ($N = 9$) consisted of rats that were given a high-fat diet for four months. The high-fat diet consisted of 2% cholesterol, 5% sugar, 0.2% propyl thiouracil, 0.5% cholic acid, and 10% lard (Oriental Yeast Co., Tokyo, Japan). The high-fat diet was given *ad libitum* in solid pellets for four months.

Serum and tissue collection

After four months of high-cholesterol diet, rats of both the groups were killed by deep ether anesthesia, and blood was collected from the inferior vena cava. The serum levels of total cholesterol and creatinine were measured by an autoanalyzer. Kidneys were removed via a midline incision, and a portion was fixed immediately in

Key words: collagen, glomerulosclerosis, hsp47, hypercholesterolemia.

Table 1. Summary of immunohistochemical staining

	Glomeruli		Tubules		Interstitium	
Antibody	Group I	Group II	Group I	Group II	Group I	Group II
α-SMA	±	+	−	−	+	++
Vimentin	++	++	±	+	±	+
Desmin	+	++	−	−	±	+
Collagen I	±	±	−	−	+	++
Collagen III	±	±	−	−	+	++
Collagen IV	+	++	+	++	±	+
HSP47	+	++	±	+	±	+
ED-1	M	S	N	M	M	S

Symbols are: (−) no staining; (±) staining involved <5%; (+) staining involved 5%–25%; (++) staining involved >25–75%; (+++) staining involved >75%. ED-1-immuno-positive monocyte/macrophage infiltration was denoted by no infiltration (N), mild infiltration (M) and severe infiltration (S). Mild infiltration was donoted when <4 ED-1-positive cells were preent in a low power field (×20) of a particular structure of the kidney; while >4 ED-1-positive cells were denoted as severe infiltration. Group I was comprised of age-matched control rats, and Group II were rats on a high cholesterol diet for 4 months.

Carnoy's solution and another portion in 10% formalin. Tissues were processed and embedded in paraffin wax. Renal sections (4 μm) were stained with hematoxylin and eosin (HE), periodic acid-Schiff stain, and periodic acid-methenamine silver stain (PAM) for histological studies.

Immunohistochemistry

Immunohistochemistry was performed on paraffin sections (4 μm) using the primary antibodies against α-smooth muscle actin (Dako Corp., Glostrup, Denmark), vimentin (Dako), desmin (Dako), type I collagen (Chemicon, Temecula, CA, USA), type III collagen (Chemicon), type IV collagen (Chemicon), ED-1 (Serotec, Oxford, UK), and hsp47 (StressGene Biotechnologies Corp., Victoria, Canada) by streptavidin-biotin-peroxidase method. The details of the immunostaining are described in earlier studies [4, 6, 7]. The immunostaining of the glomeruli, tubules, and interstitium of at least one kidney section per rat was evaluated.

Double immunostaining

This was performed to localize hsp47/α-smooth muscle actin, hsp47/desmin, and hsp47/ED-1 in the same renal section, as described earlier [4, 6–8]. Briefly, selected sections were immunostained with hsp47 by streptavidin-alkaline phosphatase method and were then counterstained with α-smooth muscle actin, desmin, or ED-1 by the streptavidin-biotin-peroxidase method. For both single and double immunostaining, as a control, primary antibodies were replaced with either mouse IgG diluted with phosphate-buffered saline (similar concentration as that of primary antibody) or were replaced with a solution containing a 10-fold excess of recombinant hsp47 (StressGene Biotechnologies Corp.) in addition to anti-hsp47 antibody.

Statistical analysis

When appropriate, the statistical analysis was assessed by the use of unpaired Student's *t*-test, calculated with StatView software (for Apple Macintosh).

RESULTS

Morphological and biochemical analysis

Long-term dietary-induced hypercholesterolemia (group II) resulted in mild glomerular hypercellularity and expansion of mesangial matrix with glomerular infiltration of foam cells and inflammatory cells. The biochemical analysis showed that compared with group I rats (68 ± 4 mg/dl), there was significantly higher level of serum cholesterol (167 ± 30 mg/dl, $P < 0.0001$) in group II rats. No significant difference in the serum creatinine level was seen in control (0.32 ± 0.11 mg/dl) and high-fat feeding rats (0.388 ± 0.06 mg/dl).

Immunolocalization of type I, type III, and type IV collagens

In the group I rat kidneys, immunostaining for type I and type III collagens was present mainly in the interstitium; type IV collagen was seen in the mesangium, glomerular basement membrane, and tubular basement membrane. In the group II rat kidneys, a slightly increased accumulation of type I and type III collagens was seen in the interstitium, whereas increased deposition of type IV collagen was mainly present in the glomerulosclerotic lesions. The pattern of immunohistochemical staining in different components of the kidney is shown in Table 1.

Immunolocalization of α-smooth muscle actin, vimentin, desmin, and ED-1

In the group I rat kidneys, α-smooth muscle actin was mostly absent in the glomeruli, but in group II rat kidneys, it was occasionally present in the glomeruli and interstitial cells. Vimentin was mostly absent in the tubular epithelial cells in the group I rat kidneys; in group II rats, tubular epithelial cells and interstitial cells were focally stained. In addition, compared with the group I rat kidney, increased expression of desmin was seen in glomerular epithelial cells in group II rat kidney, suggesting phenotypic changes of glomerular epithelial cells

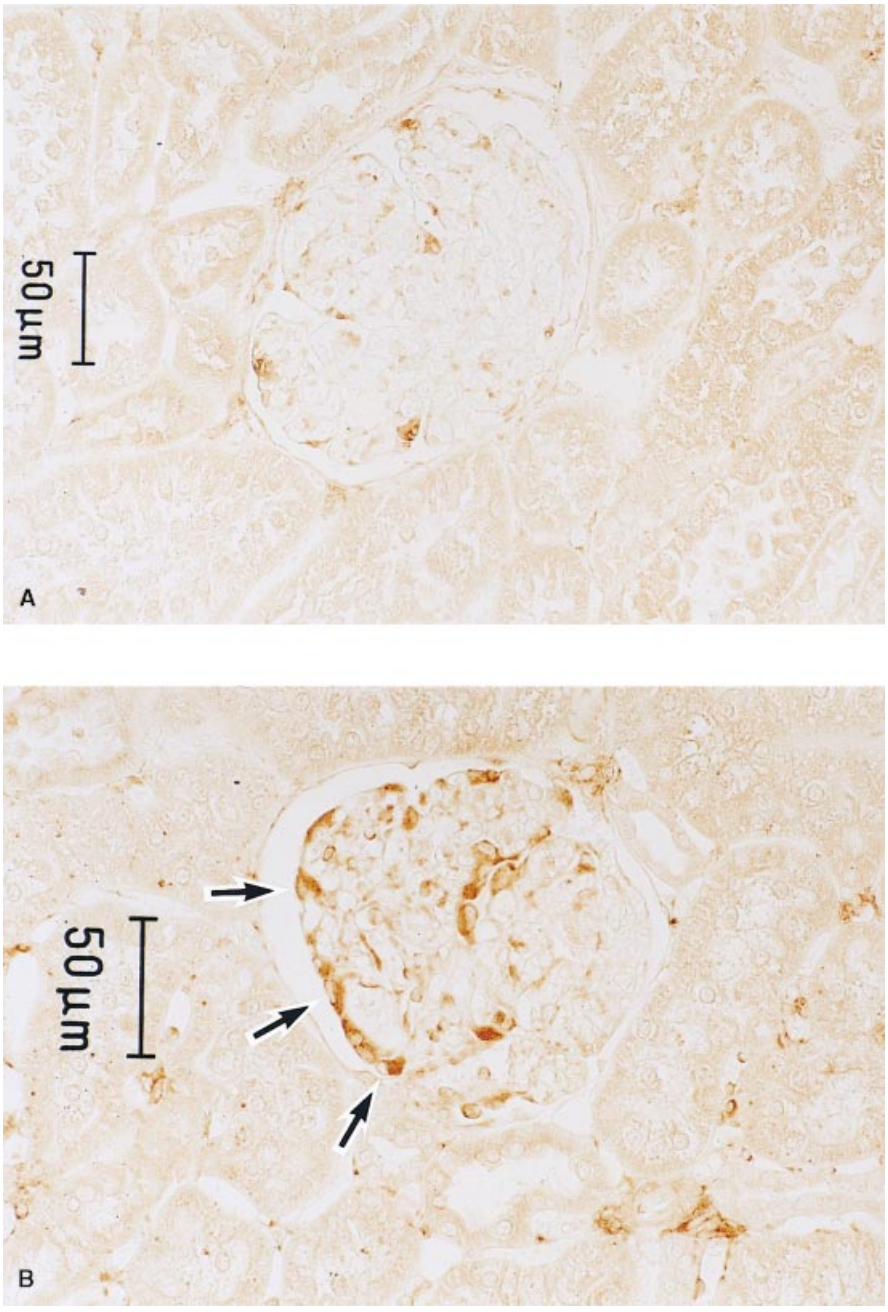

Fig. 1. Immunohistochemistry for HSP47. A control renal section shows weak expression in the glomerulus (*A*). In contrast, increased expression of HSP47 is noted in the glomerulus of high-fat–fed rat kidney (*B*); note that the expression is mostly present in the glomerular epithelial cells (arrow).

[9]. In contrast to mild infiltration of ED-1–positive monocytes/macrophages in the glomeruli of group I rat kidneys, there was severe infiltration in the group II rat kidneys.

Immunolocalization of heat shock protein 47

Compared with the group I rat kidneys (Fig. 1A), induced expression of collagen/hsp47 was noted in hypercholesterolemic rat kidneys, with a predominant expression in the glomeruli (Fig. 1B).

Double staining of heat shock protein 47 and α-smooth muscle actin, desmin, or ED-1

Most of the hsp47-expressing cells in the glomeruli coexpressed with desmin, whereas mesangial cells (α-smooth muscle actin-immunopositive) and infiltrating monocytes/macrophages (ED-1 immunopositive) were mostly negative for collagen/hsp47, suggesting that hsp47-expressing cells were mostly phenotypically altered glomerular epithelial cells [9] in the hypercholesterolemic rat kidneys.

DISCUSSION

Newly identified hsp47 has provided new insights into sclerosis/fibrosis [3–8]. Hsp47 is associated not only with *in vitro* collagen synthesis/assembly [3] but appears to be involved in the *in vivo* sclerotic/fibrotic process, as well [4–8]. Nevertheless, the possible role of hsp47 in high-fat diet-induced glomerulosclerosis was not yet known. As hsp47 seems to play a role in glomerulosclerosis in various other renal diseases [4, 7], potential exists for a pathological role of hsp47 in the high-fat diet-induced glomerular injury. Identification of hsp47-producing cells may provide additional understanding of the mechanism of high-fat diet-induced glomerulosclerosis.

An increased expression of hsp47 was noted in hypercholesterolemic rat kidneys, with predominant expression localized to glomeruli. The major finding of this study was that dietary-induced hypercholesterolemia results in phenotypical alteration of glomerular epithelial cells, as shown by desmin-immunopositivity [9]. These phenotypically altered glomerular epithelial cells were found to be the main hsp47-expressing cells in the hypercholesterolemic rat kidneys, whereas mesangial cells and infiltrating monocytes/macrophages were mostly negative for hsp47 expression. The earlier studies have shown that both human and rat podocytes are able to produce collagens and are thought to synthesize predominantly type IV collagen [10]. It is thought that the induced expression of hsp47 by phenotypically altered glomerular epithelial cells may be related to podocytic collagen synthesis, and this could subsequently play a role in the development of glomerulosclerosis in hypercholesterolemic rats.

Up to now, most of the studies have focused on the role of infiltrating glomerular macrophages and mesangial cells in the pathogenesis of early glomerular injury in rats with dietary-induced hypercholesterolemia; a possible pathological role of glomerular epithelial cells in the hypercholesterolemic rat kidneys has been relatively unexplored. In this study, we have clearly shown that the glomerular epithelial cells play an important role(s) in the early glomerular injury in rats with dietary-induced hypercholesterolemia.

ACKNOWLEDGMENTS

Part of this work was supported by the grants-in-aid for scientific research to M.S.R. (grant no. 09670192) from the Ministry of Education, Science and Culture, Japan. We are indebted to Mr. S. Nakamura and Mr. K. Hamasaki (medical students) for performing most of the immunohistochemical study. The authors thank Ms. K. Yamaguchi and S. Nakanose for their kind help in preparing paraffin sections during this study. Special thanks are due to Dr. T. Naito for helping in statistical analysis.

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